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Seasonal abundance and spatio-temporal distribution of the troglophylic harvestman *Ischyropsalis ravasinii* (Arachnida, Opiliones, Ischyropsalididae) in the Buso del Valon ice cave, Eastern Italian Prealps

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Abstract

We explore the population of the troglophilic harvestman *Ischyropsalis ravasinii* inhabiting the Buso del Valon ice cave located in the Italian Prealps. Spatial and temporal distributions of the specimens are investigated in relation to the variation of environmental abiotic conditions in the cave, such as the seasonal temperature and substrate surface typology. Our results show that *I. ravasinii* is distributed unevenly in the cave, most of individuals being present in the scree-covered section of the cave with superficial activities limited to the warm seasons only. In addition, our data suggests that the presence of a thick layer of rocky debris, together with high humidity and cold temperatures, are important limiting factors for the species. Seven additional species of harvestman are recorded in the cave, including the congeneric troglophilic species *Ischyropsalis strandi*. This is the first known record of these two troglophilic *Ischyropsalis* species coexisting within the same cave. An updated map of the distribution of *I. ravasinii* and *I. strandi* in the Italian Prealps is provided.

Keywords

Age classes, global warming, Lessinia Mountains, Northern Italy, seasonality, subterranean environment

Introduction

Harvestmen (Arachnida: Opiliones) are one of the largest orders within the class Arachnida, numbering 65 families and 6637 species (Blick and Harvey 2011; Kury et al. 2020). Harvestmen show highly diverse morphology and biology, allowing them to successfully colonize a large number of habitats including terrestrial and subterranean habitats. The genus *Ischyropsalis* C.L. Koch, 1839 (Family Ischyropsalididae Simon, 1879) contains some of most iconic European harvestmen, which are easily recognizable by their relatively large body size, massive, prominent chelicerae and dark coloration. Numbering 22 species, all geographically limited to Europe, *Ischyropsalis* species are characterized by a high level of endemism. They are often restricted to a single mountain chain (Schönhofer 2013; Schönhofer et al. 2015). They frequently show frigophilic and hygrophilic habits, having marked preferences for microhabitats with low temperature and constantly high humidity (Martens 1969; Schönhofer et al. 2015). Recent studies also suggest a direct relationship between the current distribution of Alpine *Ischyropsalis* species and the Pleistocene glaciations (Mammola et al. 2019). Although in high mountains these arachnids can be found in open or shallow humid habitats, such as scree and mossy landscapes, at lower altitudes they inhabit caves and other subterranean habitats. Thus, several *Ischyropsalis* species display an affinity for hypogean habitats and different degrees of adaptations to the subterranean life, including numerous obligate cave-dwellers (Marcellino 1982; Schönhofer et al. 2015). Despite their relevance as part of the European cave-dwelling fauna, the ecology of this genus has been scarcely explored (Martens 1969). To date, only six species have been partially analyzed in terms of ecology and life cycle, and most of the data rely on old studies carried out over 50 years ago (e.g. *I. luteipes* Simon, 1872 and *I. pyrenaea* Simon, 1872 see Juberthie 1961; *I. strandi* Kratochvil, 1936 see Juberthie 1963; *I. kollari* C.L. Koch, 1839 see Martens 1969; *I. dentipalpis* Canestrini, 1872 and *I. lithoclasica* Schönhofer & Martens, 2010 see Schönhofer and Martens 2010). For most known cave-dwelling *Ischyropsalis* no updated information concerning their life cycle, seasonality, micro-habitat preference or even area of distribution are available.

In caves, where the environmental conditions remain rather constant along the year (Badino 2010), abiotic factors represent a critical element to define the structure and composition of the subterranean communities (Howarth 1980). Local variations may deeply affect the spatial and temporal distribution of the cave-dwelling arthropods (Latella et al. 2008). In particular, frigophilic cave species have adapted to living in cold subterranean habitats characterized by low temperature and the presence of an ice or snow layer throughout the year, albeit with some seasonal variation (Iepure 2018).

Located in the Italian Prealps (Fig. 1B) at relatively low altitude, the Buso del Valon ice cave (cadaster number: 438 V/VR) has a permanent internal ice body and superficial snow (Zorzin et al. 2015). The cave hosts a well-defined assemblage of arthropods adapted to cold environments including three species of the wingless limoniid crane flies of the genus *Chionea* Dalman, 1816 (Avesani and Latella 2016; Latella et al. 2019). It also shelters a large population of the harvestman *I. ravasinii*

Hadži, 1942 (Fig. 1D), a troglophilic species endemic to the Venetian Prealps in North-East Italy. Due to its unique features, the Buso del Valon ice cave represents an ideal natural laboratory to explore the phenology and microhabitat preference of frigophilic subterranean species (Avesani and Latella 2016). With this study, we aim to complete our knowledge of the life cycle and spatio-temporal distribution of *I. ravasinii* living in cold subterranean environments in relation to the cave substratus and seasonal changes. We further plan to use data herein collected as a starting point for long-lasting studies aiming to monitor the effects of climate change on the frigophilic cave fauna and its resilience to changes in micro-habitat conditions (see e.g. Howarth, 2021).

Materials and methods

Area of study

The Buso del Valon ice cave (Fig. 1A) is located at ~1700 m a.s.l. in the Lessini Mountains of the Venetian Pre-Alps in Northern Italy (Veneto Region, Province of Verona, 45°41'32.26"N, 11°0.6"11.10"E) (Fig. 1B). The mountain chain forms a trapezium-shaped massif dominated by Mesozoic and Cenozoic limestones. These sedimentary rocks are interspersed by Cenozoic volcanic rocks and Eocenic limestone outcrops (Sauro 1973). The cave opens with a large shaft approximately 30 m in diameter and 50 m deep. It shows a vertical E-W course reaching nearly 70 m of depth at its deepest point (Fig. 1A, C). The Buso del Valon ice cave is one of the few karstic cavities of the Veneto Region with permanent cold internal temperatures ranging from -8 °C to 7 °C (Fig. 2A) and hosting a permanent ice body fed by seasonal snowfalls through the entrance. The ice inside the cave has been retreating in recent years, probably due to climate change (Latella et al. 2019).

Specimens sampling

Field collections were carried out in the cave for approximately two and half years, from July 2014 to December 2016 resulting in a consecutive temporal series of 30 months. We selected five sampling stations inside the cave (ST1–4 and DPS), located in three ecologically different areas of the cave: one at the entrance shaft base (ST1), two on the scree near the border of the internal ice layer (ST2, DPS), two near the bottom (ST3 and ST4) (Fig. 1C). Each selected area was characterized by specific combination of abiotic factors (e.g. albedo, typology and thickness of substrate; see Table 1). Four stations (ST1–4) were sampled using standard pitfall traps consisting of a glass cup with an open diameter of 10 cm filled with propylene glycol. A deep scree trap (DPS, Fig. 1E) was set approximately one meter deep inside the scree for monitoring possible seasonal vertical movement of the harvestman specimens among the debris. The DPS trap consisted of a 90 cm long PVC pipe with an inside diameter of 11 cm and several small holes (5–7 mm in diameter) drilled along its surface (see López and Oromí 2010). A 10 cm diameter plastic cup filled with propylene glycol was placed

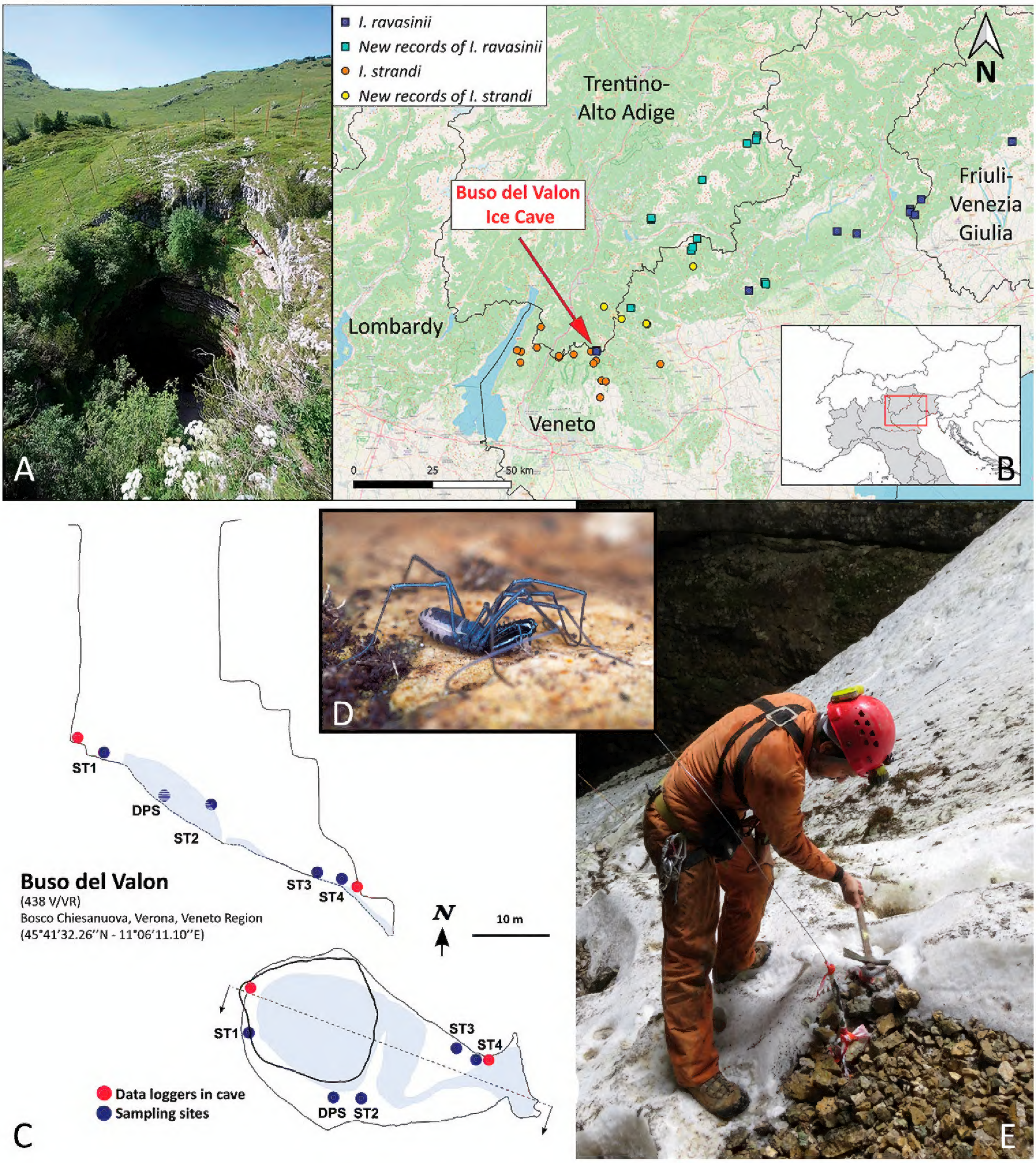

Figure 1. Location and outlines of the study area **A** entrance of the Buso del Valon ice cave **B** updated distribution of *Ischyropsalis ravasinii* and *I. strandi*: the position of the cave is highlighted by an arrow **C** transversal and horizontal sections of the Buso del Valon ice cave (modified from Zorzin et al. 2015), with the locations of the selected stations and dataloggers used in the study. The extension of the permanent ice and snow coverage inside the cave is illustrated in light blue. The external station (ST5) is not shown **D** adult male *Ischyropsalis ravasinii* **E** replacement of the deep scree trap inside the scree. Abbreviations: DPS = sampling station with deep scree trap, ST1–4 = sampling stations 1–4 with pitfall traps.

at the bottom of the pipe to collect the samples. An additional pitfall trap (ST5) was installed outside the cave near its external border acting as a control station. A bait consisting of a piece of blue mould cheese in a plastic vial was added in each trap to attract the cave-dwelling arthropods. The collected specimens were fixed in 75% ethanol for

Table 1. Position and abiotic factors of the stations used in this study. For the exact position of each sampling station see Fig. 1C.

Station	Type of trap	Position	Albedo	Surface typology	Ice/snow coverage
ST1	Superficial pitfall trap	Base of the shaft	Low	Large stones, clay and moss	No
ST2	Superficial pitfall trap	Middle cave section	Low	Thick scree	Yes-seasonal
DPS	Deep scree trap	Middle cave section	Absent	Thick scree	Yes-seasonal
ST3	Superficial pitfall trap	Bottom of the cave	Very low	Large stones and clay	No
ST4	Superficial pitfall trap	Bottom of the cave	Very low	Fissured rock	Yes-seasonal
ST5	Superficial pitfall trap	Outside of the cave	Strong	Meadow soil	No

morphological study. All specimens used in this study are preserved in the collections of the Museo di Storia Naturale of Verona, Italy.

Due to the varying albedo and temperature along the year, the permanent ice and snow layer inside the Buso del Valon ice cave shows seasonal variation in spatial coverage and thickness. Following this feature, the sampling time and consequent analysis of the data was divided into two time-frames of six months each: from mid-June to mid-December and from mid-December to mid-June. Thus, each trap was set in place for a period of 6 months before being emptied and refreshed. These periods roughly correspond to the warm and cold seasons of the year inside the cave, namely the periods of minimum and maximum temperature (Fig. 2A) and extension of the snow and ice coverage inside the cave. Two Tinytag Plus data loggers (-30 °C to +50 °C) were positioned inside the cave for a period of two years, one at the base of the shaft and one in the lower part of the cave (Fig. 1C), to record the temperature changes during the two seasons in different parts of the study area.

Stages identification and population demography

Identification of adults at species level was carried out under a stereomicroscope (Bresser Advance ICD 10-160x) according to Martens (1978). Juveniles of *I. ravasinii* were distinguished from juveniles of other species (e.g. *I. strandi*) based on the presence of eye pigmentation and length of chelicerae basal article. Previous studies have shown that the life cycle of *Ischyropsalis* species is divided into six growing instars before the adult form (Juberthie 1961; Martens 1969). Based on this information, we classified each collected specimen into one of the seven putative stages (instars IN 1–6 and adults AD) based on the length of their cephalothorax, chelicerae and cheliceral spines. These characters are considered discriminant among different instars and thus indicative of the growing stages (Martens 1969).

Adults and instar richness within each trap were calculated summing the number of individuals of *I. ravasinii* collected. Nevertheless, each trap may show unique results due to the different types of traps used (pitfalls and DPS) and slightly different collecting timeframes in different years (see Table 2). To avoid sampling bias and to make the data more directly comparable, for each trap we also defined a trapping rate (TR, *sensu* Vater 2011). Therefore, the number of specimens collected was converted

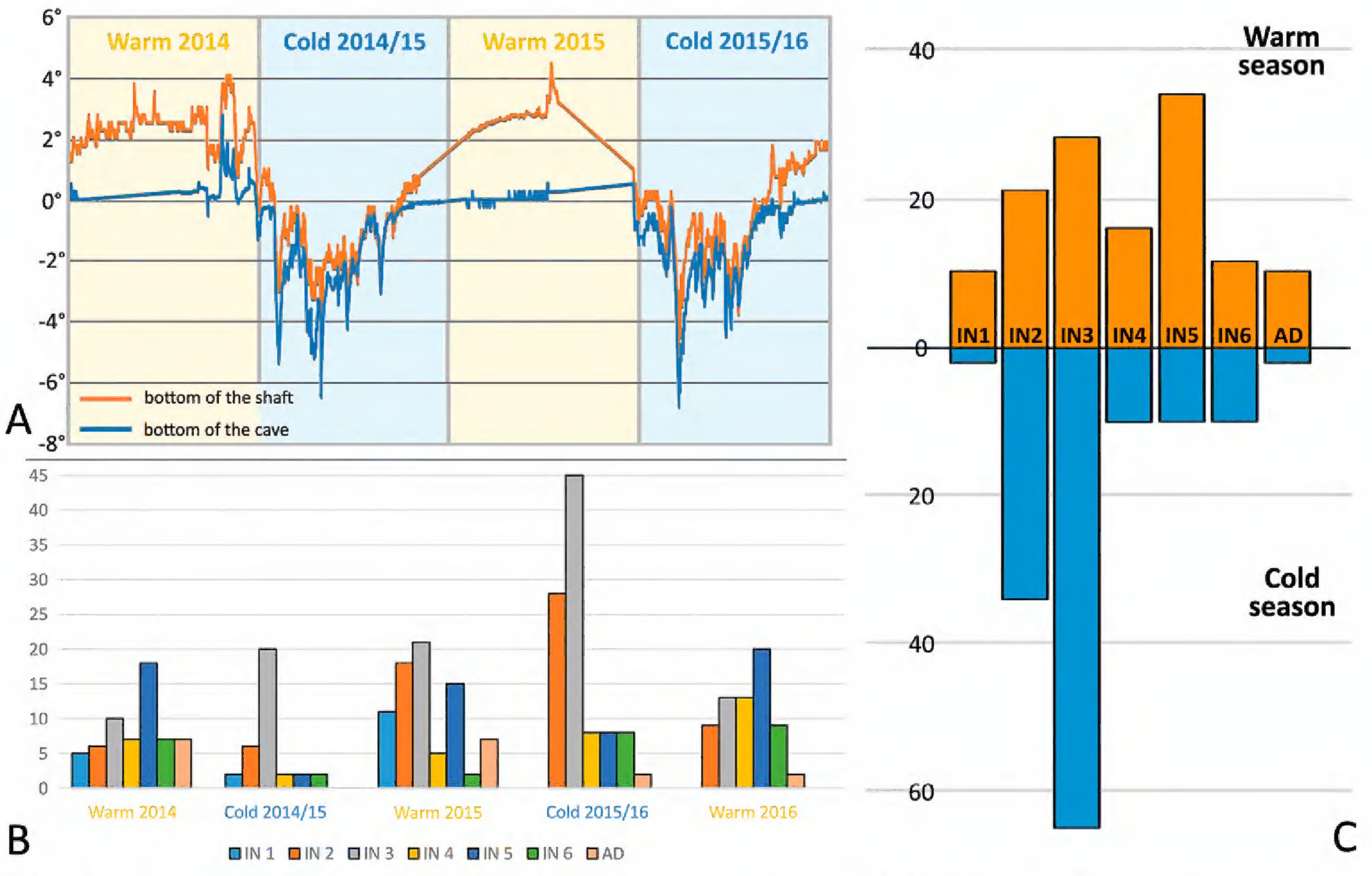

Figure 2. A temperatures recorded over two years in the Buso del Valon ice cave, for the detailed position of the dataloggers see Fig. 1A **B** seasonal instars and adult abundance collected during each separate trapping period **C** number of individuals of different growth stages collected during the warm and cold seasons. Abbreviations: AD = adults, IN 1–6 = instars 1–6.

using the following formula: TR = n° individuals/n° of days of trap activity. Statistical analyses were carried out and graphs were plotted using PAST4 and EXCEL software. The map with the updated distribution of *I. ravasinii* and *I. strandi* was constructed using QGIS version 3.4 including known records from literature and new records collected by the first author.

Captive breeding

In order to obtain supplementary information on the life cycle of *I. ravasinii*, additional specimens were collected from the artificial tunnel Galleria Vittorio Emanuele III located in the Grappa Massif, Venetian Pre-Alps. Three adults and one juvenile belonging to the 5th instar were collected in March 2020 and raised in controlled conditions for approximately 14 months. Specimens were kept in plastic boxes (size 15 × 8 × 10 cm for adults and late instars and 5 × 5 × 3 cm for the early instars) with small stones and wood sticks and a layer of peat on the bottom. The boxes were stored in a fridge with a controlled temperature of 6–8 °C. To maintain constant moisture the boxes were frequently sprayed with nebulized water. Harvestmen were fed using collembola, small flies or crickets. Hatchlings were raised until reaching the 4th instar. Adults and the juvenile collected in the field were raised until the end of their life cycle.

Table 2. List of harvestman species collected in the Buso del Valon ice cave and related stations, including numbers of individuals. Abbreviations: AD = adults, DPS = sampling station with deep scree trap, IN 1–6 = instars 1–6, ST1–5 = sampling stations 1–5 with pitfall traps.

Species	Collection period	Station	N° of specimens	Instar	Season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	1	IN 1	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	3	IN 1	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	1	IN 1	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	2	IN 2	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	4	IN 2	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	10	IN 3	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	6	IN 4	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	1	IN 4	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	14	IN 5	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	3	IN 5	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	1	IN 5	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	7	IN 6	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	6	AD (2♂, 4♀)	Warm season
Ischyropsalis ravasinii	25.IX.2014–14.XII.2014	ST2	1	AD (1♀)	Warm season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	2	IN 1	Cold season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	6	IN 2	Cold season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	20	IN 3	Cold season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	2	IN 4	Cold season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	2	IN 5	Cold season
Ischyropsalis ravasinii	14.XII.2014–27.VI.2015	DPS	2	IN 6	Cold season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	ST1	1	IN 5	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	ST2	14	IN 5	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	ST2	2	IN 6	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	DPS	11	IN 1	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	DPS	18	IN 2	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	DPS	20	IN 3	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	DPS	5	IN 4	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	DPS	7	AD (3♂, 4♀)	Warm season
Ischyropsalis ravasinii	27.VI.2015–6.XII.2015	ST3	1	IN 3	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST1	1	IN 4	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	9	IN 2	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	13	IN 3	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	9	IN 4	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	18	IN 5	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	8	IN 6	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST2	2	AD (2♀)	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST3	3	IN 4	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST3	2	IN 5	Warm season
Ischyropsalis ravasinii	27.VI.2016–8.XII.2016	ST3	1	IN 6	Warm season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	ST1	2	IN 3	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	ST2	1	IN 5	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	28	IN 2	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	42	IN 3	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	8	IN 4	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	6	IN 5	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	5	IN 6	Cold season

Species	Collection period	Station	N° of specimens	Instar	Season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	DPS	2	AD (1♂, 1♀)	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	ST4	1	IN 3	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	ST4	1	IN 5	Cold season
Ischyropsalis ravasinii	6.XII.2015–27.VI.2016	ST4	3	IN 6	Cold season
Gyas annulatus	6.XII.2015–27.VI.2016	ST4	1	1 juv.	Cold season
Gyas annulatus	25.IX.2014–14.XII.2014	ST2	1	1 juv.	Warm season
Gyas annulatus	27.VI.2015–6.XII.2015	ST3	1	1 juv.	Warm season
Gyas annulatus	27.VI.2015–6.XII.2015	ST2	1	1 juv.	Warm season
Histricostoma dentipalpe	27.VI.2016–8.XII.2016	ST1	1	AD (1 ♀)	Warm season
Histricostoma dentipalpe	27.VI.2016–8.XII.2016	ST3	2	AD (1♂, 1♀)	Warm season
Ischyropsalis strandi	27.VI.2015–6.XII.2015	ST2	2	AD (2♀)	Warm season
Ischyropsalis strandi	25.IX.2014–14.XII.2014	ST2	1	IN 2	Warm season
Ischyropsalis strandi	27.VI.2015–6.XII.2015	DPS	1	IN 1	Warm season
Lacinius horridus	27.VI.2015–6.XII.2015	ST5 (external)	1	AD (1 ♀)	Warm season
Lophopilio palpinalis	27.VI.2015–6.XII.2015	ST5 (external)	15	AD (7♂, 8♀)	Warm season
Lophopilio palpinalis	27.VI.2016–8.XII.2016	ST1	1	AD (1 ♀)	Warm season
Lophopilio palpinalis	27.VI.2016–8.XII.2016	ST3	2	AD (1♂, 1♀)	Warm season
Mitopus morio	27.VI.2015–6.XII.2015	ST5 (external)	2	AD (1♂, 1♀)	Warm season
Mitostoma sp.	6.XII.2015–27.VI.2016	DPS	1	1 juv.	Cold season
Nemastoma sp.	27.VI.2016–8.XII.2016	ST2	1	AD (1 ♀)	Warm season
Rilaena triangularis	6.XII.2015–27.VI.2016	ST2	2	AD (2♀)	Cold season

Results

Stages composition and seasonal abundance

A total of 338 specimens of *I. ravasinii* were collected inside the cave during the study period (Table 2). Among them, 18 were adults (6 males, 12 females, 5.3% of the total) and 320 were juveniles (94.7%). Juveniles belonged to the following instars: IN 1=18, IN 2=67, IN 3=110, IN 4=35, IN 5=63, IN 6=28 (Fig. 3A). No specimens of *I. ravasinii* were found in the trap located outside the cave area. Instars and adults abundance in warm and cold periods are illustrated in Fig. 2B, C. During the warm seasons, when the extension of the internal ice layer was at its minimum, a total of 205 specimens were sampled. They were partitioned into the seven stages as follows: IN 1=16, IN 2=33, IN 3=44, IN 4=25, IN 5=53, IN 6=18, AD=16 (Fig. 3A). Juveniles represented the majority of the collected specimens (92.1%), in particular the first three instars (~40% of the total) and especially the IN 5 which alone included ¼ of all the samples. Male/female sex ratio in this season was 5:11. During the cold seasons, in the periods of maximum ice extension,133 specimens were collected: IN 1=2, IN 2=34, IN 3=65, IN 4=10, IN 5=10, IN 6=10, AD=2 (Fig. 3A). Again, the majority of specimens were juveniles (98.5%), in particular those belonging to the early three instars (83.2%). The male/female sex ratio was 1:1.

Spatio-temporal distribution

Most specimens (95% of the total samples) were collected in the middle section of the cave, characterized by a thick layer of rocky debris. Samples were collected both on the surface (ST2: ~40.2%) and in the deep layers (DPS: 55%). All other stations collected a much smaller number of specimens, between 1.2% and 2.1% of the total samples (Fig. 3B).

Similar results were obtained considering the collections occurred only in the warm or the cold seasons. During the warm seasons (Fig.3C) the stations located in the scree showed the highest trapping rate both on the surface and in the deep layers (ST2 TR=0.994, DPS TR=0.449). Few specimens were collected in the other stations, near the entrance (ST1 TR=0.014) or at the bottom of the cave (ST3 TR=0.051; ST4 TR=0.00). During the cold season, all individuals were gathered in the deep layers of the scree, the deep scree trap showing the highest trapping rate (DPS TR=0.626). In contrast, only a few individuals were found on the surface, all the surface pitfall traps showing low trapping rates including in the scree (ST1 TR=0.010; ST2 TR=0.005; ST3 TR=0.00; ST4 TR=0.025) (Fig.3C).

Life cycle in captivity

Eggs were laid in captivity between late April and June 2020 always in the most humid part of the breeding boxes where several condensation drops were present. Each egg cluster contained between 10 to 20 eggs. Egg development, from deposition to hatching, required about 100 days until middle-late August. Only approximately 50% of the eggs hatched. Hatchlings needed about 11 months to reach the 4th instar, each growing stage lasting between one to three months. The juvenile of the 5th instar reached adulthood approximately three months later, in June 2020 and survived as adult for nearly one more year until May 2021. The whole life cycle is estimated to last approximately two years.

Additional notes on the opiliofauna of the Buso del Valon ice cave

In addition to *I. ravasinii*, the congeneric species *I. strandi* Kratochvil, 1936 was sampled in the study area. Only four specimens of *I. strandi* were collected during the two and half years of sampling: two adult females and two juveniles belonging to the 1st and 2nd instars, respectively (Table 2). All the specimens were found in the scree area (ST2 and DPS) during the warm seasons. The small number of individuals did not allow statistical evaluation. An updated distribution of these two *Ischyropsalis* species in the Italian Prealps is illustrated in Fig. 1B. Six additional species of harvestmen belonging to two different families and six different genera were also sampled inside the Buso del Valon cave: Fam. Nemastomidae: *Histricostoma dentipalpe* (Ausserer, 1867) (3 spec.); *Mitostoma* sp. (1 spec.); *Nemastoma* sp. (1 spec.); Fam. Phalangiidae: *Gyas annulatus* (Olivier, 1791) (4 spec.); *Lophopilio palpinalis* (Herbst, 1799) (18 spec.); *Rilaena triangularis* (Herbst, 1799) (2 spec.) (see Table 2).

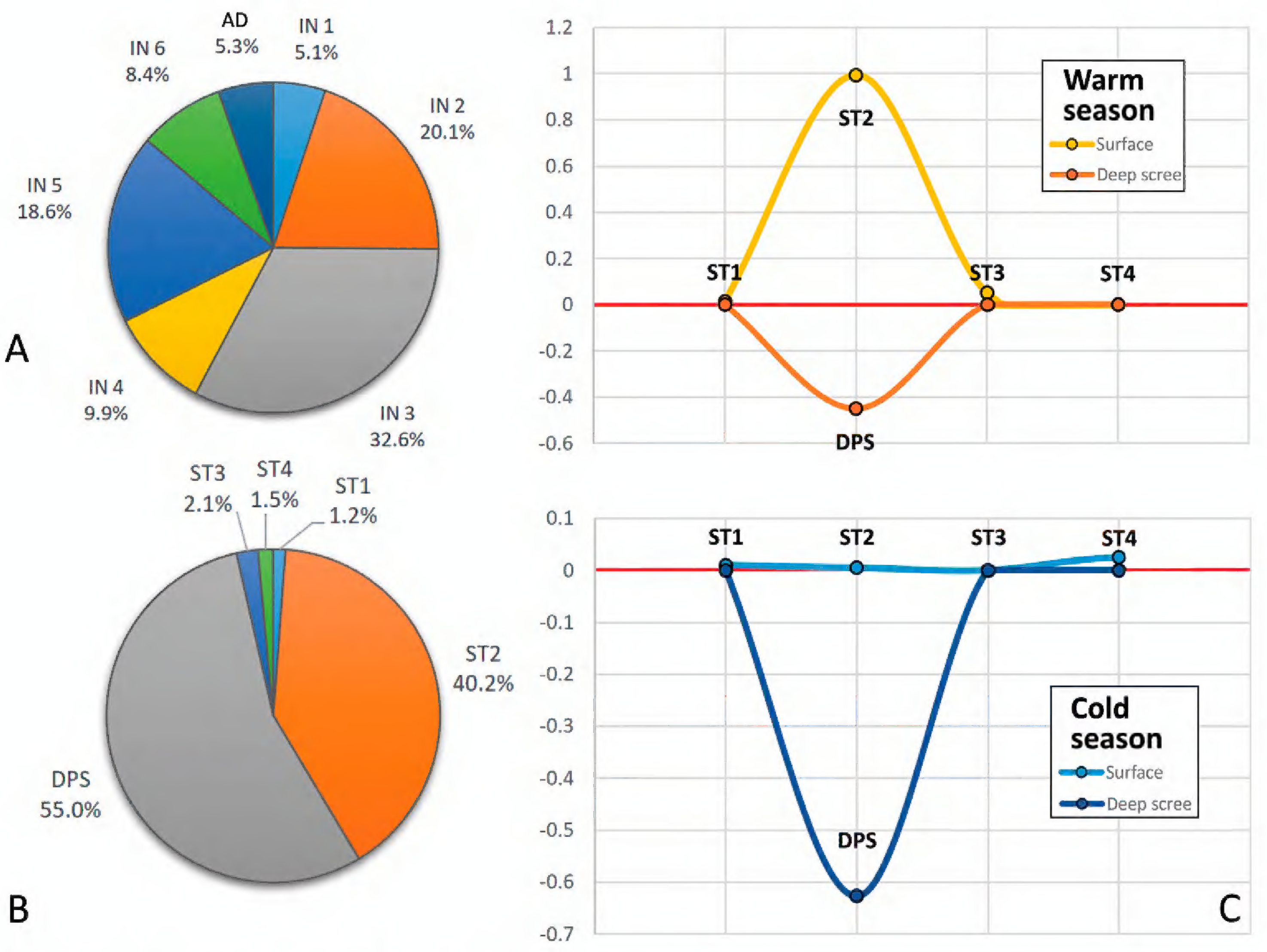

Figure 3. A percentage of instars and adult relative abundances **B** percentage of sampling relative abundances by station **C** comparison of the trapping rate (TR) of each station during the warm and cold seasons. Abbreviations: AD = adults, DPS = sampling station with deep scree trap, IN 1–6 = instars 1–6, ST1–4 = sampling stations 1–4 with pitfall traps.

Discussion

Among the harvestman fauna inhabiting the Buso del Valon ice cave, two coexisting species belonging to the genus *Ischyropsalis* were collected: *I. ravasinii* and *I. strandi*. Both the species belong to the Alpine clade *sensu* Schönhofer et al. 2015, and show a reduced distribution along the Venetian Prealps. *Ischyropsalis strandi* is endemic to the Lessini and Baldo mountains, while *I. ravasinii* extends its distribution to the East toward the Cansiglio plateau. Thus, the Buso del Valon ice cave represents the southernmost record for *I. ravasinii* (Fig 1B). The distributions of both species overlap in the western part of the Venetian Prealps (Schönhofer et al. 2015). Our data are in agreement with this finding (Fig. 1B). Despite being sympatric, to our knowledge this is the first known case of coexistence of these two troglophilic *Ischyropsalis* species within the same cave.

Ischyropsalis ravasinii appears to prosper inside the Buso del Valon ice cave, forming a large population and being the most abundant representative of the local harvestman fauna. Additionally, the lack of specimens collected outside the cave corroborates the strong affinity of *I. ravasinii* for subterranean habitats. However,

the population of *I. ravasinii* is not uniformly distributed inside the Buso del Valon ice cave. This species shows a marked preference for the micro-habitat formed by the thick scree in the central part of the cave, being most abundant near the border of the permanent ice during both the warm and cold seasons. Most of the specimens were sampled from the central part of the cave, including juveniles belonging to all six instars and all the adults. A similar distribution pattern seems to be followed by *I. strandi* although on a smaller scale. *Ischyropsalis ravasinii* is a troglophilic-hygrophilic species strictly bound to high humidity to deposit eggs (Juberthie 1964, 1965; and observations with specimens in captivity). It needs a cool environment of about 4–6 °C to remain active as observed with captive specimens. The interstitial spaces within the rocky debris may contribute to retain the suitable humidity and temperature for the harvestmen survival throughout the year. The scree likely represents the most stable micro-habitat inside the cave in both the seasons, consequently serving as an ideal habitat for egg deposition and juvenile development. Other areas of the cave pose colder or drier conditions, at least for a part of the year (Fig. 2A), or lack a coverage of debris that can be used as a refuge, thus being a less suitable habitat for this species.

Our data suggests a conspicuous difference in the seasonal distribution of *I. ravasinii* in the scree-covered area of the Buso del Valon ice cave. During the warm season, when the slightly warmer temperatures and the reduced extension of the ice coverage allows surface activity, *I. ravasinii* seems to be similarly present in both superficial and deep layers within the scree, with a preference for being close below the surface. In contrast, very few specimens were sampled on the surface during the cold season, most collections occurred in the DPS trap only. Lower surface temperatures and the presence of a larger and thicker layer of ice and snow in comparison to the warm season, most likely hinder the surface activity of *I. ravasinii* during the cold season. Such conditions may force *I. ravasinii* to move deeper into the scree where the micro-climatic conditions remain more suitable.

Martens (1969) reports that the reproductive season in *Ischyropsalis* spp. extends from spring to early summer, with egg hatching in late summer/autumn. Similar results were observed by us with *I. ravasinii* eggs hatched in captivity. Accordingly, the presence of the highest number of early instars (IN 1–3) collected in the cave during the cold season, together with the low numbers of adults, supports this hypothesis. The high percentage of the subadult IN 5 found during the warm season also suggests that the final maturation of this species occurs mainly during the warmer period. Therefore, it is likely that juvenile *I. ravasinii* need at least nine months to reach adulthood and probably even longer. Such hypotheses are in line with our experimental observations with specimens raised in captivity. After reaching their maturation, adults of some *Ischyropsalis* species may live for several months (e.g. *I. kollari*, see Martens 1969). The records of adults collected in the Buso del Valon ice cave during both the warm and cold periods also support this hypothesis. Dead adults of this species have occasionally been found in caves during the early summer months (July and August, Petri I. personal observation 2020) and captive adults have survived several months

before dying. Such findings imply that the life cycle of *I. ravasinii* extends for more than one year, possibly around two or more years, as reported for other *Ischyropsalis* species (see Juberthie 1968) and experimentally tested by us hatching and raising specimens in captivity.

Conclusions

The present study offers new data on the spatio-temporal distribution of the troglophilic harvestmen *I. ravasinii* adapted to living in cold subterranean environments. We investigated for the first time the ecological and seasonal preferences for microhabitats in *I. ravasinii* and we report additional data on its life cycle. Despite being the dominant harvestman in the cave, this species is absent in nearby cavities, including artificial tunnels, which are instead occupied by *I. strandi*. Since *I. ravasinii* seems to strongly rely on the presence of stable, humid and cool habitat, the Buso del Valon ice cave may provide refuge for this species similarly to other local frigophilic arthropods. In addition, the typology of substrate seems to play an important role in its survivability, the wide majority of individuals being collected in the scree-covered area of the cave.

Due to its strict bond with specific environmental conditions *I. ravasinii* may be strongly affected by even limited changes occurring to its habitat. Following the rise in temperatures related to climate change, and the consequent progressive reduction of the internal ice body, the conditions of the microhabitats inside the Buso del Valon ice cave are changing at a fast pace (Latella et al. 2019). Such changes may threat the species survivability similarly to what is occurring to other arthropods living in ice caves (Mammola et al. 2019; Howarth 2021). Additional studies on the ecology of *I. ravasinii* in the Buso del Valon ice cave may help us to explore how this or other frigophilic subterranean arthropods face the long-term effects of climate change.

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